Development of a New Class of Nonimidazole Histamine H_3 Receptor Ligands with Combined Inhibitory Histamine *N*-Methyltransferase Activity

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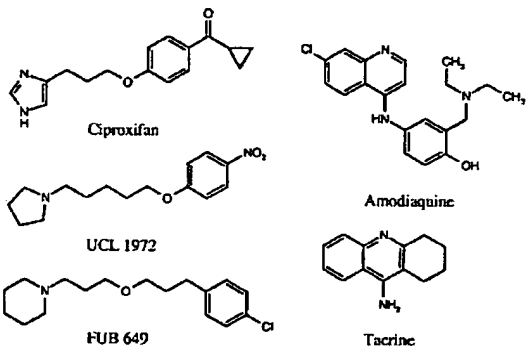
In search of novel ways to enhance histaminergic neurotransmission in the central nervous system, a new class of nonimidazole histamine H_3 receptor ligands were developed that simultaneously possess strong inhibitory activity on the main histamine metabolizing enzyme, histamine *N*-methyltransferase (HMT). The novel compounds contain an aminoquinoline moiety, which is an important structural feature for HMT inhibitory activity, connected by different spacers to a piperidino group (for H_3 receptor antagonism). Variation of the spacer structure provides two different series of compounds. One series, having only an alkylene spacer between the basic centers, led to highly potent HMT inhibitors with moderate to high affinity at human histamine H_3 receptors. The second series possesses a *p*-phenoxypropyl spacer, which may be extended by another alkylene chain. This latter series also showed strong inhibitory activity on HMT, and in most cases, the H_3 receptor affinity even surpassed that of the first series. One of the most potent compounds with this dual mode of action is 4-(4-(3-piperidinopropoxy)phenylamino)quinoline (**34**) (hH_3, K_i = 0.09 nM; HMT, IC_{50} = 51 nM). This class of compounds showed high antagonist potency and good H_3 receptor selectivity in functional assays in guinea pig on H_1, H_2, and H_3 receptors. Because of low or missing in vivo activity of two selected compounds, the proof of concept of these valuable pharmacological tools for the supposed superior overall enhancing effect on histaminergic neurotransmission failed to appear hitherto.

Introduction

Histamine is a well-known neurotransmitter that plays a crucial role in different (patho)physiological processes by interacting with four histamine receptor subtypes, named H_1, H_2, H_3,[1] and the very recently found H_4.[2] The histamine H_3 receptor is located presynaptically in the central nervous system (CNS) of many species and, in an autoreceptor function, regulates the synthesis and the release of histamine by a negative feedback mechanism.[3,4] As a heteroreceptor, this receptor subtype also modulates the release of many other neurotransmitters, e.g., glutamate, acetylcholine, serotonin, noradrenaline, and dopamine.[5] Several compounds are known to be potent histamine H_3 receptor antagonists. During recent years, new developments in histamine H_3 receptor research have included the proxifan class, e.g., ciproxifan (Chart 1), a potent and selective antagonist with high in vitro and in vivo activity,[6] and replacement of the imidazole moiety by a piperidine group to give another new class of nonimidazole histamine H_3 receptor antagonists.[7,8] The ether derivatives UCL 1972 and FUB 649, which also have high in vitro and in vivo activity, belong to this new nonimidazole class.[7,9] Cloning the histamine H_3 receptor of different species has given fresh impetus to histamine research.[10,11] Interestingly, the affinity of different antagonists varies among different species due to small differences in the amino acid sequence in the putative seven transmembrane area, thereby indirectly influencing the binding area.[12]

Chart 1. Histamine H_3 Receptor Antagonists and Inhibitors of HMT



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Histaminergic neurotransmission is controlled not only by the receptors but also by the inactivating enzyme histamine *N*-methyltransferase (HMT) [EC 2.1.1.8]. The other metabolizing enzyme for histamine, the copper-containing amine oxidase, also called diamine oxidase [EC 1.4.3.6], is important in the periphery in some species but is absent in the CNS. HMT is mainly located in glia cells and functions as the main enzyme in the CNS, metabolizing liberated histamine and using the methyl donor *S*-adenosyl-l-methionine (SAM) to form the inactive *N*-methylhistamine and *S*-adenosyl-l-homocysteine.[13] Several compounds are known to be potent inhibitors of this specific methyltransferase. Besides several antimalarial compounds such as chloroquine and amodiaquine,[14] tacrine is also one of the most potent inhibitors known so far (Chart 1).[15] Indeed, tacrine inhibits HMT up to 10 times more potently than its better known and claimed target, acetylcholinesterase.[16] Most of the HMT inhibitors have a 4-aminoquinoline moiety in common, and it seems likely that this structural feature is beneficial for high inhibitory activity.[14]

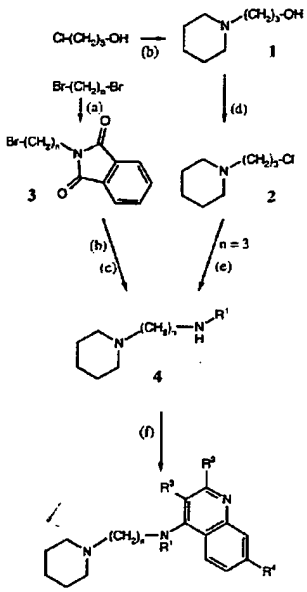
We reasoned that histaminergic neurotransmission could be greatly enhanced by a drug combining histamine-releasing properties (via H_3 autoreceptor blockade) and allowing the released amine to be protected against inactivation (via HMT inhibition). Such a novel class of drugs might have numerous therapeutic applications, namely, in the field of psychiatry and neurodegenerative diseases.[17] In this study, we present the design and synthesis of novel aminoquinoline derivatives. Their binding affinities were determined at human histamine H_3 receptors stably expressed in CHO cells. Additionally, the inhibition of HMT was investigated. To obtain potent HMT inhibitors, different 4-aminoquinolines were coupled with different spacers to a piperidine, which should increase the activity at the human histamine H_3 receptor. Additionally, to the in vitro screening at both of these targets, the selectivity of selected compounds for histamine H_3 receptors was determined vs H_1 and H_2 receptors in functional assays on isolated guinea pig organs. The oral in vivo potency was also determined in the CNS of mice by measuring the histamine level for two selected compounds in comparison to ciproxifan.

Chemistry

In the series of histamine H_3 receptor antagonists with only an alkylene spacer between the piperidine and the amino-substituted heterocycle, the synthesis started from the coupling of various α,ω-dibromalkanes with potassium phthalimide in a Gabriel synthesis to obtain the *N*-(ω-bromoalkyl)phthalimide intermediate **3** (Scheme 1).[18] Alkylation of piperidine followed by acidic cleavage of **3** resulted in the primary ω-piperidinoalkan-1-amines (**4a–h**, R = H).[19] The corresponding secondary *N*-methyl- or *N*-ethyl-ω-piperidinoalkan-1-amines (**4g–i**, R = CH_3, C_2H_5) were prepared from 1-(ω-chloroalkyl)-piperidines (**2**) by reaction with an excess of methan- or ethanamine.[20]

The reaction of the amino intermediate **4** with 4-chloroquinoline, 4,7-dichloroquinoline, or 9-chloro-1,2,3,4-tetrahydroacridine, respectively, was carried out in molten phenol. Phenol increases the reactivity of the quinolines by forming unstable phenolether intermediates with the heterocycles.[21] The phenolethers have an improved leaving group reactivity as compared to those of the halogen-substituted quinolines, thereby increasing the yields. This reaction furnished compounds **5–14**, **16–20**, and **26–29**, which were purified and crystallized as salts of oxalic acid. For the 4,7-dichloro derivatives, S_NAr replacement only took place in the 4-position due to the low electrophilic activity of the 7-position.[22]

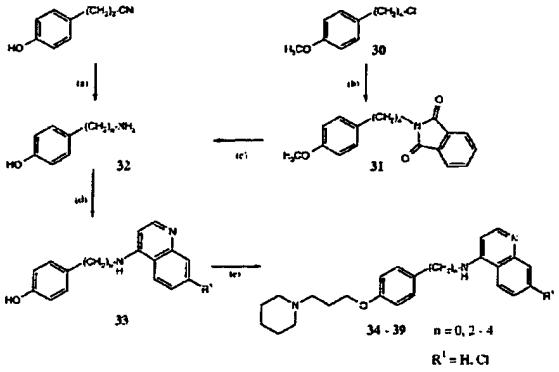
Scheme 1. Synthesis of Compounds with Alkylene Spacers Only (**1–14**, **16–20**, **26–29**)[a]



[a] Key: (a) Potassium phthalimide, KI, acetone, 50 °C, 3 d. (b) Piperidine, acetone, reflux, 12 h. (c) 6 N HCl, reflux, 12 h. (d) $SOCl_2$, THF, 50 °C, 2 h. (e) H_3CNH_2 × HCl or $H_5C_2NH_2$ × HCl, KOH, KI, H_2O, reflux, 12 h. (f) Phenol, chloroheterocycle, 140 °C, 12 h.

Compounds with a hexamethylene spacer (n = 6) (**22–25**) were obtained using different strategies. Synthesis started with coupling of 6-aminohexan-1-ol with the chloro-substituted heteroaromate in molten phenol as described before.[21] Reaction with thionyl chloride to form the corresponding chloride and additional reaction with piperidine resulted in products **22**, **23**, and **25** (not shown). The analogous 1,2,3,4-tetrahydroacridine derivative was synthesized by coupling 1,6-dibromohexane with tacrine in dimethylformamide (DMF) under basic conditions (NaH). Reaction of the alkyl bromide obtained with piperidine led to **24**. For compound **15**, this reaction sequence was followed vice versa, i.e., reaction of **2** with 9-amino-acridine in basic nonaqueous medium (not shown).[23] This Williamson synthesis analogous reaction needs dry solvents and high temperatures due to the electronic properties of the vinylogous amidine group.

Scheme 2. Synthesis of Compounds with *p*-Phenoxypropyl Spacers (**30**–**39**)[a]



[a] Key: (a) $LiAlH_4$, THF, reflux, 2 h. (b) Potassium phthalimide, KI, DMF, reflux, 12 h. (c) (i) BBr_3, CH_2Cl_2, −78 °C, 30 min; (ii) ambient temperature, 3 h; (iii) 6 N HCl, reflux, 12 h. (d) $n = 0$ (i) chloroquinoline, KI, 1 mL of 2 N HCl, EtOH, reflux, 12 h; n = 2–4 (ii) chloroquinoline, phenol, 140 °C, 12 h. (e) **2**, K_2CO_3, KI, DMF, reflux, 12 h.

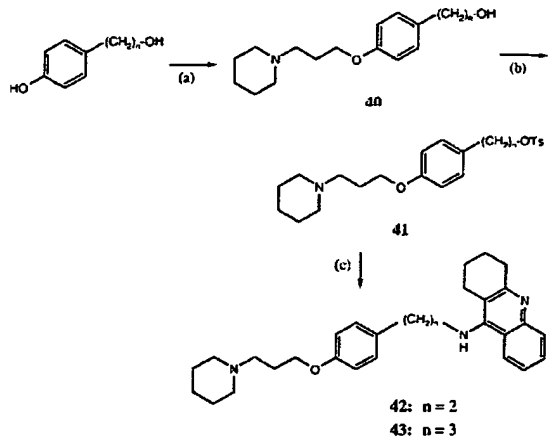
The key synthetic intermediate of histamine H_3 receptor antagonists with a *p*-phenoxypropyl spacer was 1-(3-chloropropyl)piperidine (**2**, n = 3), which was obtained by standard reaction of piperidine with 3-chloropropan-1-ol and followed by chlorination (Scheme 1).

Other key intermediates in this series were different 4-(ω-aminoalkyl)phenols and 4-aminophenol (**32**). Some of them were commercially available, and others were synthesized by different standard procedures (Scheme 2). In brief, 4-(3-aminopropyl)phenol (**32a**) was obtained by reduction of the corresponding nitrile with complex hydrides. Synthesis of 4-(4-aminobutyl)phenol (**32b**) was performed by chlorination of 4-(4-methoxyphenyl)butan-1-ol and followed by a Gabriel reaction.[18] After mild ether cleavage of **31** by boron tribromide[24] and acidic hydrolysis of the phthalimide group, the free primary amine **32b** resulted in good yields.

The amines obtained were coupled with 4-chloroquinoline or 4,7-dichloroquinoline in a halogen–amine substitution. 4-Aminophenol reacted with the heterocycles under reflux in ethanol (EtOH) and by addition of a catalytic amount of HCl. Protonation of the ring nitrogen of the quinolines increases the reactivity of the heterocycles due to higher electrophilicity of position 4.[25] Salts of quinolines **33a,b** were obtained in almost quantitative yields as well as crystallizing solids. The reaction of the other 4-(ω-aminoalkyl)phenols with the chloroquinoline compounds was carried out in phenol under the conditions mentioned above.[21] The products (**33c**–**g**) were isolated as their free bases. In the last step, the obtained intermediates reacted in a Williamson ether synthesis with 1-(3-chloropropyl)piperidine (**2**) to give **34**–**39**.[26]

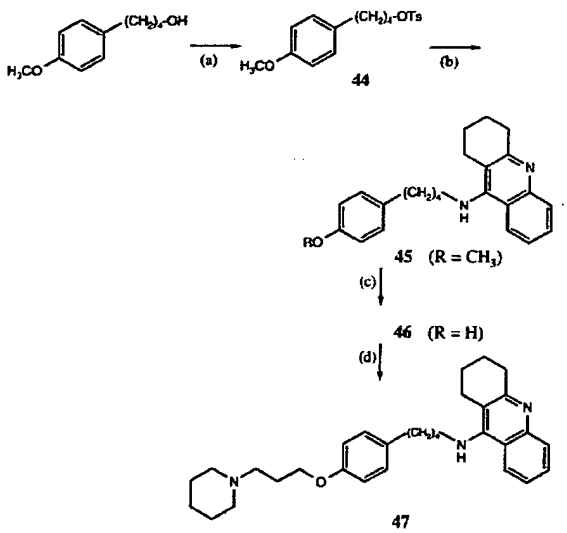
In the series of the 1,2,3,4-tetrahydroacridines, synthesis started accordingly with the reaction of 1-(3-chloropropyl)piperidine (**2**) with ω-(4-hydroxyphenyl)-alkan-1-ols in a Williamson reaction (Scheme 3). The use of potassium carbonate deprotonated the phenolic hydroxyl group only, so that the alkyl chloride reacts with the nucleophilic phenolate to form phenolether **40**.[26] No evidence of a reaction of the aliphatic hydroxyl group and the alkyl chloride was obtained. Introduction

Scheme 3. Synthesis of 1,2,3,4-Tetrahydroacridine Derivatives **42** and **43**[a]



[a] Key: (a) **2**, K_2CO_3, KI, acetone, reflux, 12 h. (b) 4-Toluenesulfonyl chloride (= Ts-Cl), pyridine, $HCCl_3$, ambient temperature, 2.5 h. (c) Tacrine, NaH, KI, DMF, 100 °C, 12 h.

Scheme 4. Synthesis of 1,2,3,4-Tetrahydroacridine Derivative **47**[a]

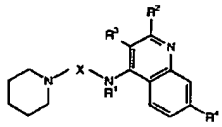


[a] Key: (a) 4-Toluenesulfonyl chloride (= Ts-Cl), pyridine, $HCCl_3$, ambient temperature, 2.5 h. (b) Tacrine, NaH, KI, DMF, 100 °C, 12 h. (c) (i) BBr_3, CH_2Cl_2, −78 °C, 30 min; (ii) ambient temperature, 3 h. (d) 3-Piperidinopropyl chloride (**2**), K_2CO_3, KI, DMF, reflux, 12 h.

of a good leaving group was performed by preparation of the corresponding *p*-toluenesulfonic acid ester (**41**),[27] which easily reacted with 9-amino-1,2,3,4-tetrahydroacridine (tacrine).[23] Side reactions, especially β-eliminations, decreased the yields of products **42** and **43**.[28]

The reaction steps mentioned before were also applied for the synthesis of compound **47** in which the order was different (Scheme 4): tosylation (**44**), nucleophilic aromatic substitution (**45**), and ether cleavage (**46**). With the longer alkylene spacer, β-elimination was not observed in contrast to the formation of **42** and **43** where the yields had accordingly been reduced to a large extent.

Table 1. Structures, Physical Data, and Pharmacological Screening Results of Heteroaromatic ω-Piperidinoalkan-1-amine Derivatives for Human Histamine H_3 Receptors and HMT Inhibition



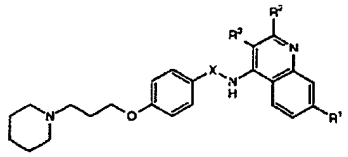
no.	X	R^1	R^2	R^3	R^4	formula	M_r	yield (%)	mp (°C)[a]	K_i (nM)[b]	IC_{50} (nM)[c]
5	$(CH_2)_2$	H	H	H	H	$C_{16}H_{21}N_3 \times 2C_2H_2O_4$	435.4	77	195	191	210 ± 30
6	$(CH_2)_2$	CH_3	H	H	H	$C_{17}H_{23}N_3 \times 2C_2H_2O_4 \times 0.5H_2O$	458.5	22	136	362	120 ± 10
7	$(CH_2)_2$	H	$-(CH_2)_4-$		H	$C_{20}H_{27}N_3 \times 2C_2H_2O_4$	489.5	34	191	19	34 ± 1
8	$(CH_2)_2$	CH_3	$-(CH_2)_4-$		H	$C_{21}H_{29}N_3 \times 2C_2H_2O_4 \times 0.75H_2O$	517.1	34	149	78	1400 ± 200
9	$(CH_2)_3$	H	H	H	H	$C_{17}H_{23}N_3 \times 2C_2H_2O_4$	449.5	34	194	85	64 ± 12
10	$(CH_2)_3$	CH_3	H	H	H	$C_{18}H_{25}N_3 \times 2C_2H_2O_4$	463.5	37	175	411	16 ± 1
11	$(CH_2)_3$	C_2H_5	H	H	H	$C_{19}H_{27}N_3 \times 2C_2H_2O_4 \times 0.25H_2O$	481.8	43	175	1130	49 ± 1
12	$(CH_2)_3$	H	H	CH_3	H	$C_{18}H_{25}N_3 \times 2C_2H_2O_4$	463.5	64	186	70	590 ± 30
13	$(CH_2)_3$	H	$-(CH_2)_4-$		H	$C_{21}H_{29}N_3 \times 2C_2H_2O_4 \times H_2O$	521.6	29	148	34	45 ± 2
14	$(CH_2)_3$	CH_3	$-(CH_2)_4-$		H	$C_{22}H_{31}N_3 \times 3C_2H_2O_4$	607.6	4	100	50	360 ± 10
15	$(CH_2)_3$	H	$-(CH)_4-$		H	$C_{21}H_{25}N_3 \times 2.8C_2H_2O_4$	571.5	11	200	188	110 ± 10
16	$(CH_2)_3$	H	H	H	Cl	$C_{17}H_{22}ClN_3 \times 2C_2H_2O_4$	483.9	45	203	378	200 ± 20
17	$(CH_2)_4$	H	H	H	H	$C_{18}H_{25}N_3 \times 2C_2H_2O_4$	463.5	33	179	90	64 ± 10
18	$(CH_2)_4$	H	H	H	Cl	$C_{18}H_{24}ClN_3 \times 2C_2H_2O_4 \times 0.5H_2O$	506.9	16	163	82	160 ± 20
19	$(CH_2)_5$	H	H	H	H	$C_{19}H_{27}N_3 \times 2C_2H_2O_4$	477.5	55	168	15	53 ± 11
20	$(CH_2)_5$	H	H	H	Cl	$C_{19}H_{26}ClN_3 \times 2C_2H_2O_4 \times 0.5H_2O$	521.0	58	169	26	140 ± 20
22	$(CH_2)_6$	H	H	H	H	$C_{20}H_{29}N_3 \times 2C_2H_2O_4 \times 0.5H_2O$	500.6	45	168	7.6	84 ± 13
23	$(CH_2)_6$	H	H	CH_3	H	$C_{21}H_{31}N_3 \times 2C_2H_2O_4 \times 0.75H_2O$	519.1	42	194	3.6	340 ± 20
24	$(CH_2)_6$	H	$-(CH_2)_4-$		H	$C_{24}H_{35}N_3 \times 2C_2H_2O_4 \times H_2O$	563.7	85	94	1.8	21 ± 2
25	$(CH_2)_6$	H	H	H	Cl	$C_{20}H_{28}ClN_3 \times 2C_2H_2O_4$	526.0	75	199	18	180 ± 10
26	$(CH_2)_7$	H	H	H	Cl	$C_{21}H_{30}ClN_3 \times 2C_2H_2O_4 \times 0.25H_2O$	544.5	60	152	9.9	120 ± 40
27	$(CH_2)_8$	H	H	H	Cl	$C_{22}H_{32}ClN_3 \times 2C_2H_2O_4$	554.0	16	151	7.8	230 ± 10
28	$(CH_2)_{10}$	H	H	H	Cl	$C_{24}H_{36}ClN_3 \times 2C_2H_2O_4$	582.1	28	151	12	220 ± 20
29	$(CH_2)_{12}$	H	H	H	Cl	$C_{26}H_{40}ClN_3 \times 2C_2H_2O_4$	610.2	9	142	32	230 ± 30
tacrine											110 ± 40

[a] Crystallized from EtOH/Et_2O. [b] [^{125}I]Iodoproxyfan binding assay to human H_3 receptor stably expressed in CHO cells.[29] [c] HMT assay on isolated enzyme from rat kidneys (mean value with standard error of the mean (SEM)).

Pharmacological Results and Discussion

Binding Assay at Cloned Human Histamine H_3 Receptors. The affinity of the compounds was determined by measuring the displacement curves of [^{125}I]-iodoproxyfan from human histamine H_3 receptors stably expressed in CHO cells.[29] In the series of compounds with an alkylene spacer only between piperidine and aromatic amine, the majority of compounds showed affinity in the nanomolar concentration range (Table 1). Affinity varied with structural modifications on the spacer length, the aromatic moiety, and the substitution pattern of the aromatic amine. For quinoline compounds with alkylene spacers from two to four methylene groups (**5**, **6**, **9**–**12**, and **16**–**18**), moderate to good affinities for histamine H_3 receptors were observed. Additional substitution of the aromatic amino group by methyl (**6**, **8**, **10**, and **14**) or ethyl residues (**11**) to tertiary amines diminished receptor affinity in comparison to that of the corresponding secondary amines. Therefore, it may be speculated that a free proton at the aromatic amino group is advantageous for the receptor–ligand interaction. Compounds with longer alkylene spacers (5–12 methylene groups, **19**, **20**, and **22**–**29**) possessed good to high affinities. In the series with a 1,6-hexylene spacer, the 7-chloroquinoline derivative **25** had more than 2 times lower affinity than

Table 2. Structures, Physical Data, and Pharmacological Screening Results of Heteroaromatic *p*-(3-Piperidinopropyloxy)phenyl Derivatives for Human Histamine H_3 Receptors and HMT Inhibition



no.	X	R^2	R^3	R^1	formula	M_r	yield (%)	mp (°C)[a]	K_i (nM)[b]	IC_{50} (nM)[c]
34		H	H	H	$C_{23}H_{27}N_3O \times 2C_2H_2O_4 \times 0.75H_2O$	555.1	50	146	0.091	51 ± 8
35		H	H	Cl	$C_{23}H_{26}ClN_3O \times 2C_2H_2O_4 \times 0.25H_2O$	580.5	36	190	0.086	310 ± 10
36	$(CH_2)_2$	H	H	H	$C_{25}H_{31}N_3O \times 2C_2H_2O_4 \times H_2O$	587.6	16	183	0.53	120 ± 10
42	$(CH_2)_2$	$-(CH_2)_4-$		H	$C_{29}H_{37}N_3O \times 2C_2H_2O_4 \times 1.25H_2O$	646.2	10	132	0.33	48 ± 6
37	$(CH_2)_2$	H	H	Cl	$C_{25}H_{30}ClN_3O \times 2C_2H_2O_4 \times H_2O$	622.1	35	150	0.53	420 ± 50
38	$(CH_2)_3$	H	H	H	$C_{26}H_{33}N_3O \times 2.5C_2H_2O_4 \times 0.5H_2O$	637.7	33	98	0.75	31 ± 5
43	$(CH_2)_3$	$-(CH_2)_4-$		H	$C_{30}H_{39}N_3O \times 2C_2H_2O_4 \times 1.25H_2O$	660.3	13	128	1.4	95 ± 16
39	$(CH_2)_4$	H	H	H	$C_{27}H_{35}N_3O \times 2.5C_2H_2O_4 \times 0.5H_2O$	651.7	34	90	1.5	73 ± 12
47	$(CH_2)_4$	$-(CH_2)_4-$		H	$C_{31}H_{41}N_3O \times 2C_2H_2O_4$	651.8	50	179	1.8	48 ± 3

[a] Crystallized from EtOH/Et_2O. [b] [^{125}I]Iodoproxyfan binding assay to human H_3 receptor stably expressed in CHO cells.[29] [c] HMT assay on isolated enzyme from rat kidneys (mean value with SEM).

22. Methyl substitution in the 2-position of the quinoline moiety (**23**) slightly increased affinity. Alkylation with a larger aliphatic moiety on the quinoline ring led to the 1,2,3,4-tetrahydroacridine derivative **24**, which showed the strongest affinity in the series of compounds with an alkylene spacer only. At the moment, it is not clear whether steric effects and/or influence on lipophilicity are the reasons for these differences. For the 7-chloroquinoline derivatives, further elongation of the spacer led to compounds with a high affinity (**26**–**28**), but a slight decrease was observed for the longest compound **29**. Comparison of different compounds having the same alkylene spacer showed that the 1,2,3,4-tetrahydroacridine moiety seems to produce affinities at histamine H_3 receptors, which are higher than those produced by the other quinolines tested.

In the series of compounds possessing a *p*-(3-piperidinopropyloxy)phenyl moiety (Table 2), high to very high affinities at human histamine H_3 receptors were determined. Compounds with an additional alkylene spacer between the phenyl group and the heteroaromatic moiety led to affinities in the range of 0.33–1.80 nM. Once more, the 1,2,3,4-tetrahydroacridine derivative **42** was more potent than the analogous quinolines (**36** and **37**). Compound **42** is more than 1.5 log units more potent than the analogous compound (**7**) in the series with an alkylene spacer only (cf. Table 1). In contrast to previous findings for three or four methylene groups, the unsubstituted quinolines (**38** and **39**) possessed higher affinities than the analogous acridines (**43** and **47**). With longer alkylene spacers between the phenyl group and the aromatic amine, the affinity clearly decreased, although it was still on a high level. This conclusion is in agreement with the results of the compounds having an alkylene spacer only (**5**–**29**) and also with compounds **34** and **35** possessing no alkylene spacer at all between the phenyl moiety and the aromatic amine. Compounds **34** and **35** had affinities at human histamine H_3 receptors in the low subnanomolar concentration range. They are the compounds with the highest affinities described in this paper and possess one of the highest H_3 receptor affinities described so far. It may be speculated that perhaps the decreased basicity of the secondary amine is responsible for the increased affinity but the number of analogous compounds is too low to draw final conclusions. Additional preliminary binding experiments using the mice H_3 receptor in an analogous experimental setting showed K_i values of 0.3, 3.1, and 1.8 nM for compounds **34**, **38**, and **47**, respectively.

In Vitro Screening for HMT Inhibitory Activity. Both series of compounds were investigated for inhibition of rat kidney HMT activity, using a new technique in which formation of the metabolite N^τ-methylhistamine is determined. Tacrine[16] was used as a reference compound. In the series of compounds with an alkylene spacer only between the piperidine and the aromatic amine (Table 1), high inhibitory potency was found. No significant differences were observed when varying the alkylene spacer (**9**, **17**, **19**, and **22**: **7**, **13**, and **24**; **16**, **18**, **20**, and **25**–**29**). Potency depends in particular on the heteroaromatic moiety. 2-Methylquinolines (**12** and **23**) were only moderately active whereas the 7-chloro-substituted derivatives (**16**, **18**, **20**, and **25**–**29**) showed a higher inhibitory activity (IC_{50} = 120–230 nM). The acridine derivative **15** showed the same activity as tacrine. Therefore, compounds having a tacrine moiety as a structural feature (**7**, **8**, **13**, **14**, and **24**) were investigated. The secondary amines **7**, **13**, and **24** (IC_{50} = 21–34 nM) showed high inhibitory potency, up to 3–5-fold more potent than tacrine. The unsubstituted secondary 4-aminoquinolines (**9**, **17**, **19**, and **22**) inhibited the enzyme in a slightly higher concentration range (IC_{50} = 53–84 nM). With the exception of compound **5**, an increased inhibitory potency was found for all quinoline derivatives having a secondary amine substituent (i.e., where R^{1-4} = H (cf. structure in Table 1)) in comparison to that of the reference compound, tacrine. Changing the aromatic amino group of the otherwise unsubstituted quinolines into a tertiary amine by alkyl substitution (**6**, **10**, and **11**) increased activity in comparison to that of their secondary analogues (**5** and **9**). Compound **10** has about 7-fold higher inhibitory activity than tacrine and is the most potent HMT inhibitor in both series. Anticipating similarly good results, the same procedure was applied to the 1,2,3,4-tetrahydroacridine derivative **7**, but methylation of **7** into **8** led to a drastic decrease in inhibitory potency.

Table 3. Activity of Selected Compounds at Histamine Receptor Subtypes

	H_3		H_2	H_1		H_3		H_2	H_1
no.	pK_i[a]	pA_2[b]	pA_2[c]	pA_2[d]	no.	pK_i[a]	pA_2[b]	pA_2[c]	pA_2[d]
34	10.0	8.5	4.4	5.3	**39**	8.8	7.1	4.7	5.5
35	10.1	8.5	4.7	5.6	**42**	9.5	8.0	4.5	5.0
36	9.3	7.8	4.9	nc[e]	**43**	8.9	7.2	4.7	5.0
37	9.3	7.6	5.3	6.1	**47**	8.7	7.5	4.7	5.1
38	9.1	7.6	5.0	5.4					

[a] [^{125}I]Iodoproxyfan binding assay to human H_3 receptor stably expressed in CHO cells.[29] [b] Functional H_3 receptor assay on guinea pig ileum (SEM ≤ 0.2).[32] [c] Functional H_2 receptor assay on guinea pig atrium (SEM ≤ 0.2).[31] [d] Functional H_1 receptor assay on guinea pig ileum (SEM ≤ 0.2).[31] [e] nc = not calculable.

In the series of compounds with a *p*-phenoxypropyl spacer, potent HMT inhibitors were found (Table 2). For the 7-chloroquinolines **35** and **37**, only moderate inhibitory potency was found, whereas all other compounds in this series were as active as or more active than tacrine. These results correlate with the findings in the series discussed before (cf. Table 1). The quinolines **34**, **36**, **38**, and **39** inhibit HMT with IC_{50} values from 31 to 120 nM. Compounds **34** and **38** were about 2 and 4 times more potent than tacrine, respectively. The 1,2,3,4-tetrahydroacridine derivatives **42** and **47** were twice as active as tacrine.

By examining all of the new compounds presented here, it is clear that potent HMT inhibitors can be found in both series. With regard to the binding at histamine H_3 receptors, the series of *p*-(3-piperidinopropyloxy)-phenyl derivatives in almost all cases possesses higher affinities than for the series with the alkylene spacer only. The second series seems to be more promising for the dual mode of action for a potential superior overall enhancing effect on histaminergic neurotransmission.

Some critical points have to be discussed concerning the pharmacological assays. Methyltransferase testing was performed on rat kidney and not on human HMT. Because both enzymes display about 84% identity[13] but also possess genetic polymorphism,[13] these problems have to be faced in a separate investigation. Another point of concern is the H_3 receptor assay. Because it is a displacement assay, it is not sure which properties these compounds really have. Therefore, additional functional experiments have been performed for compounds of the second series on guinea pig ileum.

Functional Studies on Histamine Receptors. For the series of the *p*-phenoxypropyl spacer compounds, functional H_3 receptor properties and selectivity for this receptor were measured in functional assays on isolated organs of guinea pig (Table 3).[30,31] In addition to the high affinity to human histamine H_3 receptors, all tested compounds showed high antagonist potency at the guinea pig histamine H_3 receptor. Functional antagonist potency is in good correlation with human affinity data obtained (linear regression analysis not shown, slope = 0.9596, r^2 = 0.9016, $P < 0.0001$). Compounds **34** and **35** with the highest affinity to the human histamine H_3 receptor have the strongest antagonist potency in the guinea pig ileum. Remarkably, most compounds have an affinity at human histamine H_3 receptors more than 1 order of magnitude higher than the potency at guinea pig H_3 receptors. These differences between both assays may be due to species variation and differences in the assays.

Table 4. In Vivo Potency of Selected Compounds on [^{3}H]Histamine Level in Mouse Brain Cortex

compd	dose (mg/kg)[a]	increase of [^{3}H]histamine level in the CNS (%)[b]
24	10	0 ± 4
35	10	24 ± 5
ciproxifan	3	67 ± 7

[a] Peroral to mice. [b] In comparison to control mice.

Regarding the potencies at histamine H_1 and H_2 receptors, low antagonist activity was found for all compounds (H_1, H_2: $pA_2 \leq 6.1$). The compounds were 100−10 000 times more potent at histamine H_3 receptors than at H_2 receptors, and the potencies at H_1 receptors were generally slightly higher than those at H_2 receptors. The compounds are thereby shown to be selective for H_3 receptors.

In Vivo Activity in Mouse CNS. The most promising compounds from both series were selected for in vivo testing. Because these compounds inhibit N^τ-methylhistamine formation, the usual in vivo screening assay based on formation of this metabolite in mice cannot be applied. Compounds **24** and **34** were tested for their influence on the [^{3}H]histamine level in the brain of mice, which was determined after i.v. administration of the [^{3}H]histidine precursor,[30] and changes were compared to those elicited by the standard antagonist ciproxifan (Table 4).[6] Ciproxifan strongly increased the [^{3}H]histamine level at low dosage. In contrast, compound **34** at a higher dosage increased the [^{3}H]histamine level only up to one-third as compared to ciproxifan, and even more, compound **24** did not enhance the [^{3}H]histamine level at all. Thus, proof of concept for a dual mode of action could not be demonstrated by this set. The reasons for this failure are numerous, however. First and most likely, it is based on pharmacokinetics, assuming that the compounds are badly absorbed, quickly metabolized, and/or unable to penetrate the blood−brain barrier. Second, the assay used reflects histamine turnover within CNS neurones rather than histamine levels in the extracellular synaptic space. Hence, other assays such as evaluation of the amine level in brain microdialysates or behavioral analysis should now be applied to this novel class of compounds.

Conclusion

A new class of highly potent and selective nonimidazole histamine H_3 receptor ligands have been designed. In addition to the high affinity at human H_3 receptors, these compounds possess an inhibitory activity at the histamine-metabolizing enzyme in the CNS, HMT. The resulting dual effect of an antagonist and an enzyme inhibitor should increase the levels of histamine in the synaptic space in a synergistic manner. The new class of ligands has a 4-aminoquinoline moiety as an important structural feature, valuable for HMT inhibition and advantageous for H_3 receptor affinity. Especially, the compounds with a *p*-phenoxypropyl spacer between aminoquinoline and piperidine have high affinity at human histamine H_3 receptors and high antagonist potency at the guinea pig ileum. Compound **35** is one of the most potent histamine H_3 receptor antagonists

reported so far, but it displays only moderate HMT inhibitory activity. Compound **34** combines high H_3 receptor affinity with high HMT inhibitory activity. This class of compound also presents high H_3 receptor selectivity when compared with H_1 and H_2 receptors. These new compounds might serve as novel important tools for further pharmacological investigations on histaminergic neurotransmission and its regulatory processes.

Experimental Section

Chemistry. General Procedures. Melting points were measured on an Elektrothermal IA 9000 digital apparatus (Büchi) and are uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on an Advance DPX 400 Spectrometer (Bruker 400). Chemical shifts are expressed in parts per million downfield from internal Me_4Si as a reference. ^{1}H NMR data are reported in order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; *, exchangeable by D_2O; Acr, acridinyl or 1,2,3,4-tetrahydroacridinyl; eq, equatorial; ax, axial; quin, quinolinyl; Phth, phthalimidyl; Ph, phenyl; Pip, piperazinyl, Tol, toluoyl), number of protons, and approximate coupling constant in hertz (Hz). Elemental analyses (C, H, N) were determined on a Perkin-Elmer 240 B or 240 C and are within ±0.4% of the theoretical values. Column chromatography was carried out using silica gel 40–63 μm (Macherey, Nagel & Co.). Thin-layer chromatography (TLC) control was performed under different standard conditions using silica 60-F_{254} plates, 0.2 mm thickness (Merck). Abbreviations for the solvents are the following: Et_2O, diethyl ether; EtOH; MeOH, methanol; TEA, triethylamine; THF, tetrahydrofuran.

3-Piperidinopropan-1-ol (1).[32] 3-Chloropropan-1-ol (4.72 g, 50 mmol), piperidine (8.5 g, 100 mL), and a catalytic amount of KI were refluxed in 100 mL of acetone for 12 h. The solvent was evaporated, and the product was obtained through distillation. ^{1}H NMR (CF_3COOD): δ 7.16 (br, 1H, OH), 4.07 (t, J = 5.7 Hz, 2H, CH_2OH), 3.79 (m, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.43 (t, J = 7.1 Hz, 2H, $PipCH_2$), 3.00 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.24 (m, 2H, $PipCH_2CH_2$), 1.89–2.13 (m, 5H, 2Pip-3-H, Pip-4-H_{eq}, 2Pip-5-H), 1.64 (m, 1H, Pip-4-H_{ax}). Anal. ($C_8H_{17}NO$) C, H, N.

1-(3-Chloropropyl)piperidine (2).[9] To a solution of **1** (6.1 g, 50 mmol) in 20 mL of THF was added $SOCl_2$ (6.5 g, 55 mmol) under cooling with water. The mixture was stirred for 2 h at 50 °C. After the excess of $SOCl_2$ was removed, the residue was crystallized in EtOH/Et_2O. ^{1}H NMR ([D_6]DMSO (dimethyl sulfoxide)): δ 10.53* (s, 1H, NH^+), 3.73 (t, J = 6.4 Hz, 2H, CH_2Cl), 3.38–3.41 (m, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.06–3.10 (m, 2H, $PipCH_2$), 2.80–2.89 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.19 (m, 2H, CH_2CH_2Cl), 2.16–2.23 (m, 5H, 2Pip-3-H, Pip-4-H_{eq}, 2Pip-5-H), 1.75–1.81 (m, 1H, Pip-4-H_{ax}). Anal. ($C_8H_{16}ClN$ × HCl) C, H, N.

General Procedure for *N*-(ω-Bromoalkyl)phthalimides (3). A solution of the α,ω-dibromoalkane (40 mmol) in 30 mL of acetone was heated with potassium phthalimide (3.7 g, 20 mmol) and a catalytic amount of KI at 50 °C for 3 days. The precipitation was filtered, the solvent was evaporated, and the residue was purified by column chromatography using gradient eluation petrolether/CH_2Cl_2 (1:0 to 2:3).

***N*-(7-Bromoheptyl)phthalimide (3a).**[33] mp 34 °C. ^{1}H NMR ([D_6]DMSO): δ 7.82–7.88 (m, 4H, 4Phth-H), 3.56 (t, J = 7.1 Hz, 2H, CH_2Phth), 3.50 (t, J = 6.7 Hz, 2H, $BrCH_2$), 1.77 (m, 2H, $BrCH_2CH_2$), 1.59 (m, 2H, CH_2CH_2Phth), 1.28–1.38 (m, 6H, $BrCH_2CH_2(CH_2)_3$). Anal. $C_{15}H_{18}BrNO_2$.

***N*-(8-Bromoctyl)phthalimide (3b).**[34] mp 54–55 °C. ^{1}H NMR ([D_6]DMSO): δ 7.81–7.88 (m, 4H, 4Phth-H), 3.56 (t, J = 7.1 Hz, 2H, CH_2Phth), 3.50 (t, J = 6.7 Hz, 2H, $BrCH_2$), 1.77 (m, 2H, $BrCH_2CH_2$), 1.58 (m, 2H, CH_2CH_2Phth), 1.27–1.37 (m, 8H, $BrCH_2CH_2(CH_2)_4$). Anal. $C_{16}H_{20}BrNO_2$.

***N*-(10-Bromodecyl)phthalimide (3c).**[35] mp 62–63 °C. ^{1}H NMR ([D_6]DMSO): δ 7.82–7.88 (m, 4H, 4Phth-H), 3.56 (t, J = 7.1 Hz, 2H, CH_2Phth), 3.50 (t, J = 6.7 Hz, 2H, $BrCH_2$), 1.77 (m, 2H, $BrCH_2CH_2$), 1.58 (m, 2H, CH_2CH_2Phth), 1.24–1.37 (m, 12H, $BrCH_2CH_2(CH_2)_6$). Anal. $C_{18}H_{24}BrNO_2$.

***N*-(12-Bromododecyl)phthalimide (3d).**[36] mp 63.5–64 °C. ^{1}H NMR ([D_6]DMSO): δ 7.82–7.88 (m, 4H, 4Phth-H), 3.56 (t, J = 7.1 Hz, 2H, CH_2Phth), 3.51 (t, J = 6.6 Hz, 2H, $BrCH_2$), 1.77 (m, 2H, $BrCH_2CH_2$), 1.58 (m, 2H, CH_2CH_2Phth), 1.22–1.37 (m, 16H, $BrCH_2CH_2(CH_2)_8$). Anal. $C_{20}H_{28}BrNO_2$.

General Procedure for ω-Piperidinoalkan-1-amines (4a–f). Piperidine (3 mL) and **3** (10 mmol) were stirred in 40 mL of acetone under reflux for 12 h. The solvent was evaporated, and the residue was dissolved in ethyl acetate. The organic layer was washed with 2 N K_2CO_3, extracted with 2 N HCl, and after the aqueous layer was washed and alkalyzed, extraction followed with CH_2Cl_2. The solvent was removed under reduced pressure, and the residue was refluxed with 6 N HCl for 12 h. The acid layer was washed with CH_2Cl_2, alkalyzed, and extracted with CH_2Cl_2. After the solvent was evaporated, the product was purified by column chromatography.

3-Piperidinopropan-1-amine (4a).[36] bp 101 °C at 33 mbar [eluent: CH_2Cl_2/MeOH (8:2, ammonia atmosphere)]. ^{1}H NMR ([D_6]DMSO): δ 8.22* (s, 1H, NH), 3.34–3.38 (m, 4H, Pip-2-H_{eq}, Pip-5-H_{eq}, CH_2NH_2), 2.81–2.88 (m, 4H, Pip-2-H_{ax}, Pip-6-H_{ax}, $PipCH_2$), 2.05 (m, 2H, $PipCH_2CH_2$), 1.67–1.85 (m, 5H, 2Pip-3-H, Pip-4-H_{eq}, 2Pip-5-H), 1.40 (m, 1H, Pip-4-H_{ax}). Anal. $C_8H_{18}N_2$.

4-Piperidinobutan-1-amine (4b).[37] bp 103–105 °C at 16 mbar [eluent: CH_2Cl_2/MeOH (8:2, ammonia atmosphere)]. ^{1}H NMR ([D_6]DMSO): δ 8.05* (s, 1H, NH), 3.31 (m, 4H, Pip-2-H_{eq}, Pip-6-H_{eq}, CH_2NH_2), 2.93 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.79 (t, J = 7.4 Hz, 2H, $PipCH_2$), 1.55–1.76 (m, 10H, 2Pip-3-H, 2Pip-4-H, 2Pip-5-H, $PipCH_2(CH_2)_2$). Anal. $C_9H_{20}N_2$.

7-Piperidinoheptan-1-amine (4c). Oil [eluent: CH_2Cl_2/MeOH/TEA (90:10:5)]. ^{1}H NMR (CF_3COOD): δ 6.80* (s, 2H, NH_2), 3.71 (m, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.31 (m, 2H, $PipCH_2$), 3.20 (m, 2H, CH_2NH_2), 2.99 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 1.87–2.11 (m, 8H, $PipCH_2CH_2$, $CH_2CH_2NH_2$, 2Pip-3-H, 2Pip-5-H), 1.50–1.62 (m, 8H, $PipCH_2CH_2(CH_2)_3$, 2Pip-4-H). Anal. $C_{12}H_{26}N_2$.

8-Piperidinooctan-1-amine (4d). Oil [eluent: CH_2Cl_2/MeOH/TEA (90:10:5)]. ^{1}H NMR ([D_6]DMSO): δ 7.92* (s, 1H, NH), 2.91 (m, 6H, 2Pip-2-H, 2Pip-5-H, $PipCH_2$), 2.75 (t, J = 7.5 Hz, 2H, CH_2NH_2), 1.50–1.73 (m, 10H, 2Pip-3-H, 2Pip-4-H, 2Pip-5-H, $PipCH_2CH_2$, $CH_2CH_2NH_2$), 1.27 (m, 8H, $PipCH_2CH_2(CH_2)_4$). Anal. $C_{13}H_{28}N_2$.

10-Piperidinodecan-1-amine (4e). Oil [eluent: CH_2Cl_2/MeOH/TEA (90:5:5)]. ^{1}H NMR ([D_6]DMSO): δ 3.04 (m, 4H, 2Pip-2-H, 2Pip-6-H), 2.90 (t, J = 8.1 Hz, 2H, $PipCH_2$), 2.75 (t, J = 7.5 Hz, 2H, CH_2NH_2), 1.50–1.72 (m, 10H, 2Pip-3-H, 2Pip-4-H, 2Pip-5-H, $PipCH_2CH_2$, $CH_2CH_2NH_2$), 1.27 (m, 12H, $PipCH_2CH_2(CH_2)_6$). Anal. $C_{15}H_{32}N_2$.

12-Piperidinodecan-1-amine (4f). [Eluent: CH_2Cl_2/MeOH/TEA (90:5:5)]. ^{1}H NMR ([D_6]DMSO): δ 2.50 (m, 4H, 2Pip-2-H, 2Pip-6-H), 2.26 (m, 2H, $PipCH_2$), 2.17 (t, J = 7.4 Hz, 2H, CH_2NH_2), 1.23–1.49 (m, 26H, 2Pip-3-H, 2Pip-4-H, 2Pip-5-H, $PipCH_2(CH_2)_{10}$). Anal. $C_{17}H_{36}N_2$.

General Procedure for *N*-Alkyl ω-Piperidinoalkan-1-amine (4g–i). A solution of methan- or ethanamine × HCl (30 mmol), KOH (2.8 g, 50 mmol), 1-(ω-chloroalkyl)piperidine × HCl (7.5 mmol), and a catalytic amount of KI in 30 mL of water was refluxed for 12 h. The product was extracted with CH_2Cl_2 and purified by column chromatography using CH_2Cl_2/MeOH (95:5, ammonia atmosphere).

***N*-Methyl 2-Piperidinoethan-1-amine (4g).**[38] bp 54–58 °C at 1 mbar. ^{1}H NMR (CF_3COOD): δ 3.77–3.91 (m, 4H, CH_2NHCH_3, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.09–3.23 (m, 7H, $PipCH_2$, Pip-2-H_{ax}, Pip-6-H_{ax}, $NHCH_3$), 2.03–2.10 (m, 5H, 2Pip-3-H, Pip-4-H_{eq}, 2Pip-5-H), 1.64 (m, 1H, Pip-4-H_{ax}). Anal. $C_8H_{18}N_2$.

***N*-Methyl 3-Piperidinopropan-1-amine (4h).**[39] bp 95 °C at 20 mbar. ^{1}H NMR (CF_3COOD): δ 3.77 (m, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.35 (m, 4H, CH_2NHCH_3, $PipCH_2$), 3.13 (m, 5H, Pip-2-H_{ax}, Pip-6-H_{ax}, $NHCH_3$), 2.51 (m, 2H, $PipCH_2CH_2$), 1.83–2.14 (m, 5H, 2Pip-3-H, Pip-4-H_{eq}, 2Pip-5-H), 1.64 (m, 1H, Pip-4-H_{ax}). Anal. $C_9H_{20}N_2$.

$MgSO_4$ 1.2, NaH_2PO_4 1.3, $NaHCO_3$ 25.0, D-glucose 5.5, and choline chloride 0.001, aerated with 95% O_2/5% CO_2 (V/V) and kept at 37 °C. Mepyramine (1 μM) was present throughout the experiment to block ileal H_1 receptors. After an equilibration period of 1 h with washings every 10 min, the preparations were stimulated for 30 min with rectangular pulses of 15 V and 0.5 ms at a frequency of 0.1 Hz. Viability of the muscle strips was monitored by addition of the H_3 receptor agonist (*R*)-α-methylhistamine (100 nM), which caused a relaxation of the twitch response of more than 50 up to 100%. After wash out, reequilibration, and 30 min field stimulation, a cumulative concentration−response curve to (*R*)-α-methylhistamine (1−1000 nM) was constructed. Subsequently, the preparations were washed intensively and reequilibrated for 20−30 min in the absence of the antagonist under study. During the incubation period, the strips were stimulated continuously for 30 min. Finally, a second concentration−response curve to (*R*)-α-methylhistamine was obtained.[29,32] The rightward displacement of the curve to the H_3 receptor agonist evoked by the antagonist under study was corrected with the mean shift monitored by daily control preparations in the absence of antagonist.

Histamine H_3 Receptor Antagonist Potency In Vivo in Mice.[30] In vivo testing was performed after peroral administration of the compounds as a methylcellulose suspension to male Swiss mice as described by Garbarg et al.[30] After 90 min, [^{3}H]histidine was injected i.v., 10 min later animals were sacrified, and the brain was dissected out and homogenized in 10 volumes of ice-cold perchloric acid (0.4 M). [^{3}H]Histamine levels were determined after an ion exchange chromatographic purification and liquid scintillation spectrometry. By treatment with 3 mg/kg of ciproxifan, the maximal [^{3}H]histamine level was obtained. Results were related to the basal [^{3}H]histamine level determined in control mice.

In Vitro Screening at Other Histamine Receptors. Selected compounds were screened for histamine H_2 receptor activity on the isolated spontaneously beating guinea pig right atrium as well as for H_1 receptor activity on the isolated guinea pig ileum by standard methods described by Hirschfeld et al.[31] Each pharmacological test was performed at least in triplicate, but the exact type of interaction has not been determined in each case. The values given represent the mean.

Inhibition of HMT. HMT was isolated from rat kidneys and purified by a procedure developed by Bowsher et al.[45] with slight modification.[46] Compounds were incubated in different concentrations at 37 °C in a 20 mM phosphate buffer, pH 8.0, together with histamine (1 μM final concentration) and SAM (20 μM final concentration) in the presence of HMT. After 20 min, the reaction was stopped by addition of ice-cold perchloric acid (0.4 N final concentration). The *N*$^\pi$-methylhistamine formed was measured by a specific enzyme immunoassay. From the curve [concentration of inhibitor]·[*N*$^\pi$-methylhistamine concentration] is calculated the IC_{50} value for each compound.

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